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High resolution structure of a potassium channel – antibody Fab complex

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Beamline: X25

Introduction: Potassium channels control the electric potential across cell membranes by catalyzing the rapid, selective diffusion of K^+ ions down their electrochemical gradient [1]. The process of dehydration, transfer and re-hydration of a K^+ ion is catalyzed by the channel's selectivity filter. To study the mechanism of ion translocation in potassium channels, it is necessary to solve the K^+ channel structure at a high resolution that would reveal protein chemistry and the ordered water molecules around K^+ ions with high accuracy.

Methods and Materials: We raised monoclonal antibody against the KcsA potassium channel, and purified the Fab fragment. A KcsA-Fab complex with a stoichiometry of one Fab fragment per channel subunit was produced and crystallized in space group I4. Data collections were conducted at X25 on two types of frozen crystals: KcsA-Fab complex crystallized in high concentration of K^+ ions diffracted X-rays to 2.0 Å (cell: a = 155.33, b = 155.33, c = 76.27, $\alpha = \beta = \gamma = 90^\circ$), and the complex crystallized in low concentration of K^+ ions diffracted to 2.3 Å (cell: a = 155.29, b = 155.29, c = 75.74, $\alpha = \beta = \gamma = 90^\circ$). Phases were solved by molecular replacement [2,3]. The high-K^+ structure was refined to R_f and R_w of 23.3% and 21.8%, and the low-K^+ structure was refined to R_f and R_w of 23.5% and 21.8%, respectively.

Results: In these two structures, ions within the selectivity filter were well resolved. The structures also revealed ordered water molecules around the K^+ ions near the entryways to the filter. These two structures allowed us to address the mechanisms of K^+ ion hydration and dehydration, and the response of the selectivity filter the changing ionic environment imposed by channel gating.

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